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REMARKS/ARGUMENTS

Claims 1-10, 12-28, 30-36, 38-43, 45-50, 52-57, 59-63, and 95-157 are currently pending and under examination in the application.

Claims 11, 29, 37, 44, 51, 58, and 64-94 have been cancelled, and claims 1, 12, 16, 27, 30, 35, 38, 43, 45, 50, 52, 57, and 59 have been amended, without prejudice or disclaimer, solely for the purpose of expediting patent prosecution in accordance with the U.S. Patent Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). Applicants reserve the right to present any cancelled subject matter in a copending application.

Claims 95-157 have been added to more fully encompass Applicants' invention.

Amended claims 1, 27, 35, 43, 50, and 57 include recitation of "orally administering" and "administration of SAHA," and transpose recitation of "or a pharmaceutically acceptable salt or hydrate thereof" (see, *inter alia*, page 15, lines 23-28; and page 24, lines 2-8 of the application as originally filed).

Amended claims 12, 16, 30, 38, 45, 52, and 59 have been corrected to include proper claim dependencies.

New claim 95 recites: "wherein the composition is continuously administered once daily at a dose of 400 mg" (see, *inter alia*, page 11, lines 11-14 and 25-27; and page 65, lines 31-32 of the application as originally filed).

New claim 96 recites: "wherein the composition is administered twice daily at a dose of 100 mg or 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 64, lines 29-32 to page 65, lines 1-30 of the application as originally filed).

New claim 97 recites: "wherein the composition is administered twice daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24; and page 64, lines 29-32 to page 65, lines 1-5 of the application as originally filed).

New claim 98 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 21-22 of the application as originally filed).

New claim 99 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 11-16 and 21-22 of the application as originally filed).

New claim 100 recites: "wherein the composition is administered three times daily at a dose of 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 65, lines 28-30 of the application as originally filed).

New claim 101 recites: "wherein the composition is administered three times daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24 of the application as originally filed).

New claim 102 recites: "wherein the composition is administered twice daily at a dose of 300 mg for 3 days per week" (see, *inter alia*, page 66, lines 13-15 of the application as originally filed).

New claim 103 recites: "wherein the composition is continuously administered once daily at a dose of 400 mg" (see, *inter alia*, page 11, lines 11-14 and 25-27; and page 65, lines 31-32 of the application as originally filed).

New claim 104 recites: "wherein the composition is administered twice daily at a dose of 100 mg or 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 64, lines 29-32 to page 65, lines 1-30 of the application as originally filed).

New claim 105 recites: "wherein the composition is administered twice daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24; and page 64, lines 29-32 to page 65, lines 1-5 of the application as originally filed).

New claim 106 recites: "wherein the composition is administered three times daily at a

dose of 100-250 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 21-22 of the application as originally filed).

New claim 107 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 11-16 and 21-22 of the application as originally filed).

New claim 108 recites: "wherein the composition is administered three times daily at a dose of 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 65, lines 28-30 of the application as originally filed).

New claim 109 recites: "wherein the composition is administered three times daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24 of the application as originally filed).

New claim 110 recites: "wherein the composition is administered twice daily at a dose of 300 mg for 3 days per week" (see, *inter alia*, page 66, lines 13-15 of the application as originally filed).

New claim 111 recites: "wherein the composition is continuously administered once daily at a dose of 400 mg" (see, *inter alia*, page 11, lines 11-14 and 25-27; and page 65, lines 31-32 of the application as originally filed).

New claim 112 recites: "wherein the composition is administered twice daily at a dose of 100 mg or 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 64, lines 29-32 to page 65, lines 1-30 of the application as originally filed).

New claim 113 recites: "wherein the composition is administered twice daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24; and page 64, lines 29-32 to page 65, lines 1-5 of the application as originally filed).

New claim 114 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 21-22 of the

application as originally filed).

New claim 115 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 11-16 and 21-22 of the application as originally filed).

New claim 116 recites: "wherein the composition is administered three times daily at a dose of 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 65, lines 28-30 of the application as originally filed).

New claim 117 recites: "wherein the composition is administered three times daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24 of the application as originally filed).

New claim 118 recites: "wherein the composition is administered twice daily at a dose of 300 mg for 3 days per week" (see, *inter alia*, page 66, lines 13-15 of the application as originally filed).

New claim 119 recites: "wherein the composition is continuously administered once daily at a dose of 400 mg" (see, *inter alia*, page 11, lines 11-14 and 25-27; and page 65, lines 31-32 of the application as originally filed).

New claim 120 recites: "wherein the composition is administered twice daily at a dose of 100 mg or 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 64, lines 29-32 to page 65, lines 1-30 of the application as originally filed).

New claim 121 recites: "wherein the composition is administered twice daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24; and page 64, lines 29-32 to page 65, lines 1-5 of the application as originally filed).

New claim 122 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 21-22 of the application as originally filed).

New claim 123 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 11-16 and 21-22 of the application as originally filed).

New claim 124 recites: "wherein the composition is administered three times daily at a dose of 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 65, lines 28-30 of the application as originally filed).

New claim 125 recites: "wherein the composition is administered three times daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24 of the application as originally filed).

New claim 126 recites: "wherein the composition is administered twice daily at a dose of 300 mg for 3 days per week" (see, *inter alia*, page 66, lines 13-15 of the application as originally filed).

New claim 127 recites: "wherein the composition is continuously administered once daily at a dose of 400 mg" (see, *inter alia*, page 11, lines 11-14 and 25-27; and page 65, lines 31-32 of the application as originally filed).

New claim 128 recites: "wherein the composition is administered twice daily at a dose of 100 mg or 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 64, lines 29-32 to page 65, lines 1-30 of the application as originally filed).

New claim 129 recites: "wherein the composition is administered twice daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24; and page 64, lines 29-32 to page 65, lines 1-5 of the application as originally filed).

New claim 130 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 21-22 of the application as originally filed).

New claim 131 recites: "wherein the composition is administered three times daily at a

dose of 100-250 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 11-16 and 21-22 of the application as originally filed).

New claim 132 recites: "wherein the composition is administered three times daily at a dose of 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 65, lines 28-30 of the application as originally filed).

New claim 133 recites: "wherein the composition is administered three times daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24 of the application as originally filed).

New claim 134 recites: "wherein the composition is administered twice daily at a dose of 300 mg for 3 days per week" (see, *inter alia*, page 66, lines 13-15 of the application as originally filed).

New claim 135 recites: "wherein the composition is continuously administered once daily at a dose of 400 mg" (see, *inter alia*, page 11, lines 11-14 and 25-27; and page 65, lines 31-32 of the application as originally filed).

New claim 136 recites: "wherein the composition is administered twice daily at a dose of 100 mg or 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 64, lines 29-32 to page 65, lines 1-30 of the application as originally filed).

New claim 137 recites: "wherein the composition is administered twice daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24; and page 64, lines 29-32 to page 65, lines 1-5 of the application as originally filed).

New claim 138 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 21-22 of the application as originally filed).

New claim 139 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg for 14 days followed by 1 week without dose administration" (see, *inter*

alia, page 11, lines 11-16 and 21-22 of the application as originally filed).

New claim 140 recites: "wherein the composition is administered three times daily at a dose of 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 65, lines 28-30 of the application as originally filed).

New claim 141 recites: "wherein the composition is administered three times daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24 of the application as originally filed).

New claim 142 recites: "wherein the composition is administered twice daily at a dose of 300 mg for 3 days per week" (see, *inter alia*, page 66, lines 13-15 of the application as originally filed).

New claim 143 recites: "[a] method of treating Myelodysplastic Syndrome (MDS) in a subject, said method comprising the step of orally administering to the subject a total daily dose of up to 800 mg of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure: [structure] or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat MDS in said subject" (see, *inter alia*, Example 5, page 83, lines 1-33 to page 84, lines 1-12; page 11, lines 6-7; page 12, lines 2-9; and page 24, lines 2-8 of the application as originally filed).

New claim 144 recites: "wherein said composition is administered once-daily, twice-daily or three times-daily" (see, *inter alia*, page 15, lines 8-11 of the application as originally filed).

New claim 145 recites: "wherein said composition is administered once daily at a dose of 200-600 mg" (see, *inter alia*, page 11, lines 17-18 of the application as originally filed).

New claim 146 recites: "wherein said composition is administered twice daily at a dose of 200-400 mg" (see, *inter alia*, page 11, lines 18-19 of the application as originally filed).

New claim 147 recites: "wherein said composition is administered twice daily at a dose

of 200-400 mg intermittently" (see, *inter alia*, page 11, lines 11-16 and 18-19; and page 65, lines 8-10 of the application as originally filed).

New claim 148 recites: "wherein said composition is administered three times daily at a dose of 100-250 mg" (see, *inter alia*, page 11, lines 21-22 of the application as originally filed).

New claim 149 recites: "wherein the composition is continuously administered once daily at a dose of 400 mg" (see, *inter alia*, page 11, lines 11-14 and 25-27; and page 65, lines 31-32 of the application as originally filed).

New claim 150 recites: "wherein the composition is administered twice daily at a dose of 100 mg or 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 64, lines 29-32 to page 65, lines 1-30 of the application as originally filed).

New claim 151 recites: "wherein the composition is administered twice daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24; and page 64, lines 29-32 to page 65, lines 1-5 of the application as originally filed).

New claim 152 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 21-22 of the application as originally filed).

New claim 153 recites: "wherein the composition is administered three times daily at a dose of 100-250 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 11-16 and 21-22 of the application as originally filed).

New claim 154 recites: "wherein the composition is administered three times daily at a dose of 200 mg intermittently" (see, *inter alia*, page 11, lines 11-14 and 23-24; and page 65, lines 28-30 of the application as originally filed).

New claim 155 recites: "wherein the composition is administered three times daily at a dose of 200 mg for 14 days followed by 1 week without dose administration" (see, *inter alia*, page 11, lines 14-16 and 23-24 of the application as originally filed).

New claim 156 recites: "wherein the composition is administered twice daily at a dose of 300 mg for 3 days per week" (see, *inter alia*, page 66, lines 13-15 of the application as originally filed).

New claim 157 recites: "wherein SAHA is the active ingredient in said composition" (see, *inter alia*, page 9, lines 16-20; page 70, lines 19-26; and page 71, lines 23-31 to page 72, lines 1-5 of the application as originally filed).

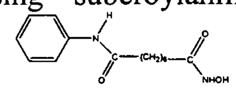
These amendments and added claims are supported by the application as originally filed, and do not constitute new matter. Support is shown in parentheses, above. Entry of these amendments is respectfully requested.

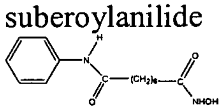
35 U.S.C. §103(a)

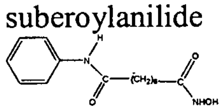
Claims 1-65 and 69-94 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over DiMartino, U.S. Patent No. 6,905,669 ("DiMartino"; Office Action, pages 2-3). Claims 66-68 have also been rejected under this section as allegedly unpatentable over DiMartino in view of WO 98/55449 and WO 95/31977. The Examiner states that it would have been obvious for one of skill in the art to modify the timing and dosage amounts reported in DiMartino to obtain the presently claimed methods (Office Action, page 4). Applicants respectfully traverse this rejection.

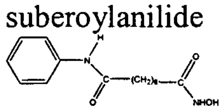
For rejection under 35 U.S.C. §103, there must be some suggestion or motivation to modify the cited reference to obtain the claimed invention. *See* MPEP §2143. The mere fact that a cited reference can be modified does not render the claims obvious unless the prior art suggests the desirability of the modification. *See In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1990); MPEP §2143.01. Further, the cited reference must teach or suggest all of the claim limitations. MPEP §2142. The Office may not disregard express claim limitations or distill down to the "gist" of the invention. *See W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1548 (Fed. Cir. 1983). Finally, the Office must consider the unexpected results of the claimed invention as evidence of nonobviousness. *In re Dillon*, 919 F.2d 688, 692-693 (Fed. Cir. 1990); MPEP §2144.08(II)(B).

Applicants note that claims 64-94 have been cancelled herein without prejudice or disclaimer (see above). In addition, as presented herein, claims 1, 27, 35, 43, 50, 57, and new claim 143 read as follows:

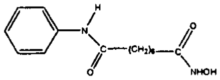
1. A method of treating leukemia in a subject, said method comprising the step of *orally administering* to the subject a *total daily dose of up to about 800 mg* of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure:  or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat leukemia in said subject. (Emphasis added).

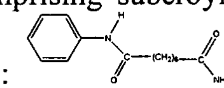
27. A method of treating Acute Myeloid Leukemia (AML) in a subject, said method comprising the step of *orally administering* to the subject a *total daily dose of up to about 800 mg* of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure:  or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat AML in said subject. (Emphasis added).

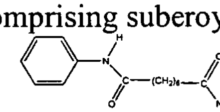
35. A method of treating Acute Lymphocytic Leukemia (ALL) in a subject, said method comprising the step of *orally administering* to the subject a *total daily dose of up to about 800 mg* of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure:  or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat ALL in said subject. (Emphasis added).

43. A method of treating Chronic Lymphocytic Leukemia (CLL) in a subject, said method comprising the step of *orally administering* to the subject a *total daily dose of up to about 800 mg* of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure:  or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat CLL in said subject. (Emphasis added).

50. A method of treating Chronic Myeloid Leukemia (CML) in a subject, said method comprising the step of *orally administering* to the subject a *total daily dose of up to about 800 mg* of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure:

 or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat CML in said subject. (Emphasis added).

57. A method of treating Hairy Cell Leukemia in a subject, said method comprising the step of *orally administering* to the subject a *total daily dose of up to about 800 mg* of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure:  or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat Hairy Cell Leukemia in said subject. (Emphasis added).

143. A method of treating Myelodysplastic Syndrome (MDS) in a subject, said method comprising the step of *orally administering* to the subject a *total daily dose of up to 800 mg* of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA) represented by the structure:  or a pharmaceutically acceptable salt or hydrate thereof, and a pharmaceutically acceptable carrier or diluent, wherein administration of SAHA is effective to treat MDS in said subject. (Emphasis added).

The remaining claims ultimately depend from claims 1, 27, 35, 43, 50, 57, or 143. Thus, all of the current claims encompass methods of treatment by orally administering to the subject a total daily dose of up to about 800 mg of a pharmaceutical composition comprising suberoylanilide hydroxamic acid (SAHA).

In contrast to the instant claims, DiMartino fails to report any specific dosages or dosage schedules for oral administration of SAHA. Instead, DiMartino reports dosages only for *intravenous administration*, and only for HDAC inhibitors *depsipeptide*, *phenylbutyrate*, and *arginine butyrate* (see, *e.g.*, DiMartino, column 22, lines 10-30). It is well known that depsipeptide, phenylbutyrate, and arginine butyrate are structurally disparate from SAHA. It is further known that intravenous and oral delivery can require widely differing dosages due to low bioavailability of oral formulations (see, *e.g.*, abstract from Schellens reference, Exhibit 2, discussed below). Yet, the Examiner has provided no indication of how artisans could use DiMartino's intravenous dosages for structurally unrelated drugs to derive the specifically

claimed oral dosages and schedules for SAHA. DiMartino fails to teach or suggest these claim elements, and no suggestion or motivation has been provided to modify DiMartino to obtain the instant claims. *See* MPEP §2143.01; *In re Mills*, 916 F.2d 680, 682 (Fed. Cir. 1990).

Moreover, the claimed methods of oral administration produce *unexpected half-life* for SAHA given the available knowledge in the art. *See* MPEP §716.02(a). Applicants show that oral administration of SAHA results in significantly longer half-life for the drug as compared to intravenous administration (see, *e.g.*, page 79, lines 9-11; Tables 2 and 3; and Figure 10 of the instant application). Oral SAHA produces a two- to three-fold increase in half-life as compared to intravenous delivery (see, *e.g.*, page 79, lines 9-11; Tables 2 and 3; and Figure 10 of the instant application; and Kelly *et al.*, 2005, *J. Clin. Oncol.* 23:3923-3931; Exhibit 1, page 3929, right column). The increased half-life from oral SAHA produces sustained histone acetylation in patients (Exhibit 1, page 3930, left column). The half-life for oral SAHA is surprising in view of prior cancer drugs, many of which exhibit short half-life (*e.g.*, high levels extraction in the gut wall or liver) from oral dosage (see, *e.g.*, Schellens *et al.*, 2000, *Eur. J. Pharm. Sci.* 12:103-110; Exhibit 2, page 103, right column to page 104, left column).

Thus, even if DiMartino could be modified and applied against the current claims (which Applicants still contest), the claimed methods of oral administration show unexpected advantages which could not have been known from the cited publication. *See U.S. v. Adams*, 383 U.S. 39, 50-51 (1966); MPEP §2144.08(II)(B).

Applicants conclude that the cited reference fails to teach or suggest the claimed oral doses and dosing schedules; no suggestion or motivation has been provided to obtain the specifically claimed oral doses and dosing schedules; and oral SAHA shows surprising half-life duration. For at least these reasons, DiMartino cannot make obvious the current claims. From cancellations of claims 66-68, the rejections over DiMartino combined with WO 98/55449 and WO 95/31977 are made moot. Reconsideration of the instant claims is respectfully requested.

CONCLUSION

A favorable action on the merits is respectfully requested. If any discussion of this Amendment would be deemed helpful, the Examiner is encouraged to contact the undersigned at

the telephone number provided below. Applicants believe no further fee is due at this time; however, the Commissioner is authorized to charge any additional fees that may be due, or to credit any overpayment, to the undersigned's account, Deposit Account No. **50-0311**, Reference No. **24852-501 CIP4**, Customer No. **35437**.

Date: January 23, 2006

Respectfully submitted,

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ORIGINAL REPORT

Phase I Study of an Oral Histone Deacetylase Inhibitor, Suberoylanilide Hydroxamic Acid, in Patients With Advanced Cancer

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Terms in blue are defined in the glossary, found at the end of this issue and online at www.jco.org.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
To determine the safety, dosing schedules, pharmacokinetic profile, and biologic effect of orally administered histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) in patients with advanced cancer.

Patients and Methods
Patients with solid and hematologic malignancies were treated with oral SAHA administered once or twice a day on a continuous basis or twice daily for 3 consecutive days per week. Pharmacokinetic profile and bioavailibity of oral SAHA were determined. Western blots and enzyme-linked immunosorbent assays of histones isolated from peripheral-blood mononuclear cells (PBMNCs) pre and post-therapy were performed to evaluate target inhibition.

Results
Seventy-three patients were treated with oral SAHA and major dose-limiting toxicities were anorexia, dehydration, diarrhea, and fatigue. The maximum tolerated dose was 400 mg qd and 200 mg bid for continuous daily dosing and 300 mg bid for 3 consecutive days per week dosing. Oral SAHA had linear pharmacokinetics from 200 to 600 mg, with an apparent half-life ranging from 91 to 127 minutes and 43% oral bioavailability. Histones isolated from PBMNCs showed consistent accumulation of acetylated histones post-therapy, and enzyme-linked immunosorbent assay demonstrated a trend towards a dose-dependent accumulation of acetylated histones from 200 to 600 mg of oral SAHA. There was one complete response, three partial responses, two unconfirmed partial responses, and 22 (30%) patients remained on study for 4 to 37+ months.

Conclusions
Oral SAHA has linear pharmacokinetics and good bioavailability, inhibits histone deacetylase activity in PBMNCs, can be safely administered chronically, and has a broad range of antitumor activity.

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INTRODUCTION

Histone deacetylases (HDACs) are enzymes that regulate chromatin structure and function through the catalysis of the removal of the acetyl modification from lysine residues of histones.[1] While the base sequence of DNA provides the fundamental code for proteins, post-translational modification of proteins plays a major role in the control of gene transcription. The amino acid tails of the core nucleosomal histones are subject to post-translational modifications by acetylation of lysines, methylation of lysines and arginines, phosphorylation of serines, and ubiquitination of lysines. The most extensively studied post-translational modification of histones is the acetylation of

lysine. The opposing activities of HDACs and histone acetyl transferases (HATs) regulate the balance of acetylation of histones. HDACs are also involved in reversible acetylation of nonhistone proteins, such as p53, tubulin, various transcription factors, and other proteins.[2-4]

HDAC inhibitors have been shown to cause cultured, transformed cells to undergo growth arrest, terminal differentiation, apoptosis, or autophagic cell death.[1] These agents act selectively in altering the transcription of relatively few of the expressed genes (generally 2% to 10% of expressed genes are increased or decreased in their rate of transcription).[5-8] HDAC inhibitors have been found to be additive and even synergistic with a number of anticancer agents including radiation, anthracyclines, flavopiridol, imatinib, proteasome inhibitors, and all *trans*-retinoic acid in blocking the proliferation or inducing apoptosis in tumor cells in culture.[1]

Inhibitors of HDAC represent a new class of targeted anticancer agents. A number of structural classes of HDAC inhibitors have been developed and are in clinical trials, including short chain fatty acids (the benzamides [MS-275]), the cyclic peptide, depsipeptide (FK-228), and suberoylanilide hydroxamic acid (SAHA).[1] We have previously reported the results of a phase I clinical trial of the HDAC inhibitor, SAHA, administered intravenously (IV) to patients with solid or hematologic malignancy.[9] This trial showed that SAHA caused an accumulation of acetylated histones in normal and malignant cells post-therapy, was well tolerated, and had antitumor activity, indicating the agent was effective in reaching and inhibiting its targets. These data, supported by in vitro studies, suggested that daily administration of SAHA may improve the therapeutic benefit, and an oral formulation was developed to improve the feasibility of daily administration. We now report the results of the phase I clinical study with orally administered SAHA in patients with advanced cancers.

PATIENTS AND METHODS

Patient Eligibility

Adult patients with solid tumors or hematologic malignancies who had failed or relapsed from standard therapy were eligible. All patients were required to have a Karnofsky performance status ≥ 70% and adequate hepatic and renal function. Solid tumor patients were required to have platelet count ≥ 100,000 cells/μL and WBC ≥ 3,500 cells/μL, and patients with hematologic malignancies were required to have an absolute neutrophil count ≥ 500 cells/μL and a platelet count ≥ 25,000 cells/μL. All patients with solid tumors were required to have radiographic evidence of measurable or nonmeasurable metastatic disease. Patients were required to have recovered from the acute toxicities of any prior therapy, and no chemotherapy, radiation therapy, or other investigational anticancer therapy for a minimum of 4 weeks before initiating the protocol. Leukemia patients could have received conventional cytotoxic therapy and lymphoma patients could receive steroids up to 2 weeks before starting therapy.

Patients with clinically significant cardiac or pulmonary disease, active CNS disease, or active infection were not eligible. Pregnant women and lactating females were excluded. The study was approved by the Memorial Sloan-Kettering Cancer Center (New York, NY) institutional review board, and all patients signed an informed consent.

Trial Design and Treatment Plan

Oral SAHA was provided by Aton Pharma, Inc (Tarrytown, NY) as 200-mg capsules and later in the study a 50-mg capsule was available. The gelatin capsules contain SAHA, and standard pharmaceutical excipients (microcrystalline cellulose, sodium croscarmellose, and magnesium stearate). The starting dose was one tenth the maximum tolerated dose (MTD) in nonrodent species (90 mg/m^2/d or approximately 200 mg daily). Due to the initial availability of only the 200-mg capsule, and preclinical data predicting poor bioavailability of SAHA, a fixed dosing schedule was used. There were eight cohorts of oral SAHA studied. SAHA was given daily at 200 mg qd, 400 mg qd, 600 mg qd, or 400 mg bid (cohorts 1 to 4). The dose was planned to be escalated to 1,200 mg bid, however, dose-limiting toxicity (DLT) was encountered at 400 mg bid. The protocol was amended to evaluate 200 mg bid and 300 mg bid dose levels (cohorts 5 and 6) and an intermittent dosing schedule of 300 mg bid and 400 mg bid for three consecutive days weekly (cohorts 7 and 8). Dose escalation proceeded independently in patients with solid tumors and hematologic malignancies. To minimize exposing patients to subtherapeutic treatment, hematologic patients were enrolled in cohorts 2 to 5. Patients were instructed to take oral SAHA at home in a fasting state, but later were allowed to take with food. Patients recorded the date and time of the ingestion of the oral SAHA capsule(s), and a pill count was performed to evaluate compliance and accountability of the study drug.

A treatment cycle was 4 weeks of therapy. DLT was defined as: grade 4 neutropenia or thrombocytopenia; grade 3 neutropenia with fever (solid tumor patients only); and grade 3 or 4 nonhematologic toxicity (solid tumor and hematologic malignancy patients) during the first cycle of therapy. A treatment delay due to toxicity that lasted longer than 1 week was also considered a DLT. At least three patients were entered per cohort and individual cohorts were expanded to six patients after the development of one DLT. MTD was defined as the highest dose with an observed incidence of DLT in no more than one of six patients treated at a dose level. At least six and as many as 20 patients would be treated at the MTD in the solid tumor and hematologic malignancy groups. Toxicities were evaluated by the National Cancer Institute Common Toxicity Criteria (version 2.0).

Patient Evaluation

The pretreatment evaluation included history and physical examination (H&P), Karnofsky performance status, a complete CBC, hepatic and renal function tests, coagulation profile (prothrombin time/partial thromboplastin time), urinalysis, and chest x-ray. A pregnancy test was obtained in women with childbearing potential. Appropriate tumor markers were obtained in patients with prostate or breast cancer. Imaging studies included a chest, abdominal, and pelvic computed tomography (CT) scan, magnetic resonance imaging scan, and positron emission tomography or bone scan as clinically indicated. All patients had a baseline ECG and further cardiac work-up if indicated.

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Patients were evaluated weekly with H&P and laboratory tests (CBC, hepatic\renal function, prothrombin time/partial thromboplastin time) and urinalysis during the first 8 weeks of therapy. Tumor markers were repeated every 2 weeks and imaging studies every 8 weeks. An ECG was obtained before every cycle of therapy. If the patient was on study longer than 8 weeks, H&P and laboratory tests were performed every other week for 8 weeks and then monthly thereafter with imaging studies performed every 4 months.

All patients were assessed for toxicity and response if they received any treatment. In patients with measurable disease, standard WHO phase II response criteria[10] were utilized and radiographs underwent a blinded review by a radiologist. The Cheson criteria were employed for patients with lymphoma and leukemia.[11,12]

Pharmacokinetics Studies. Pharmacokinetic (PK) studies were performed during the first cycle of therapy in 44 patients. To assess the absolute bioavailability of oral SAHA, the initial 18 patients received a 2-hour infusion of intravenous SAHA that was equivalent to the assigned oral dose of SAHA on day 1 of therapy. Blood (10 mL) was drawn at time 0, 30, 60, 115, 135, 150, 180, 210, 240, 300, and 360 minutes following the intravenous infusion of SAHA. After 1 week without receiving SAHA, patients started the oral SAHA (day 8). On day 8 (fasting), patients fasted (no food or beverage other than water for 2 hours before the ingestion of SAHA) before the administration of oral SAHA, and on day 9 (fed), all patients received a standardized meal (a bagel with cream cheese or butter, a pint of orange juice, and a cup of coffee with milk and sugar) 30 minutes before the ingestion of SAHA capsule(s). PK studies were performed on days 8 and 9 and on day 15 or 22. Four patients that were on the oral SAHA therapy for more than 6 months repeated the PK study. PK study consisted of drawing 10 mL of heparinized blood at time 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, and 600 minutes following oral SAHA ingestion.

Blood samples were placed on ice and refrigerated until they were centrifuged to separate the plasma. The plasma (3 to 5 mL) was transferred to a labeled conical 15 mL polypropylene screw top tube and stored at -20°C. Determination of SAHA concentrations in plasma samples was conducted in the Pharmacology Analytical Laboratory at the Memorial Sloan-Kettering Cancer Center. A liquid chromatography-mass spectrometry method was employed. A 450-μL aliquot of thawed plasma was mixed with 50 μL of d_5-SAHA (internal standard). The plasma was filtered through 0.45-μm cellulose acetate filters (Costar, Corning, NY) by centrifugation at 3,000 $\times$ g for 12 minutes. A 200-μL aliquot of filtrate was transferred to an autosampler vial. An injection volume of 30 μl was directly injected through a Prospekt 2 in-line solid phase extraction system (Spark-Holland, Emmen, the Netherlands), which consisted of a high-pressure eluter and an automated cartridge exchanger. After washing the cartridge with water, the mobile phase was changed to methanol: 0.1%:formic acid (1:1, vol:vol) at a flow rate of 0.4 mL/min, and passed over to a Reliance SBC8 4 mm $\times$ 80 mm column (Agilent Technologies, Wilmington, DE). The eluant was assayed using a diode array UV/VIS detector (at 240 nm), and an Agilent mass spectrometer detector (Agilent Technologies). The mass spectrometer detector was operated in the atmospheric pressure chemical ionization–positive mode. The masses monitored were 265 for SAHA and 270 for d_5-SAHA. The detection limit for SAHA was about 15 ng/mL, and the response was linear from 15 ng/mL to 1,000 ng/mL ($r^2 > 0.99$).

The area under the plasma concentration time curve (AUC) was calculated using the linear trapezoid method. The terminal elimination rate constant, λz, was calculated as the negative of the slope of the terminal log-linear portion of the plasma concentration time curve. Total plasma clearance (CL) and volume of distribution (Vz) were calculated using standard formulas without correcting for bioavailability. The bioavailability after oral administration (F) was calculated for each patient at a given dose on the day of sampling ($F = AUC_{oral}/AUC_{IV} \times Dose_{oral}/Dose_{IV}$). All PK calculations were performed using noncompartmental methods with WinNonLin version 3.1 (Pharsight Corp, Mountain View, CA).

Correlative Studies. Effect of SAHA on histone acetylation in mononuclear cells was assessed by Western blotting and enzyme-linked immunosorbent assay (ELISA). Peripheral blood (10 to 30 mL) was obtained in heparinized tubes at pretreatment, 2 hours postinfusion and between 2 to 10 hours after ingestion of SAHA capsule(s). Histones were isolated from the peripheral-blood mononuclear-cells (PBMNCs) and acetylated histone H3 was evaluated by Western blot analysis as previously described.[9]

For the ELISA, 50 to 100 ng of histone extract was passively adsorbed to triplicate wells of Immulon microtiter plates (VWR, West Chester, PA) and incubated overnight at 4°C. After washing with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBS-Tween), the plates were blocked for 1 hour at room temperature using PBS-Tween containing 5% nonfat milk and 1% goat serum. Total and acetylated histone H3 levels were then quantified by using an antihistone H3 rabbit polyclonal (Abcam, Cambridge, MA) or an antiacetylated histone H3 rabbit polyclonal (Upstate Biotechnology, Lake Placid, NY), respectively. The primary antibodies were diluted in PBS-Tween containing goat serum (0.5%) and added to the appropriate microtiter plates. After 1 hour at room temperature, the plates were washed with PBS-Tween and a horseradish peroxidase–conjugated goat antirabbit secondary antibody (Bio-Rad, Hercules, CA) was added. The plates were incubated at room temperature for 1 hour and then extensively washed with PBS-Tween. Horseradish peroxidase signals were visualized using the TMB peroxidase substrate kit (Bio-Rad) according to manufacturer's instructions. Normalized histone H3 acetylation levels for each sample were derived by dividing the acetylated histone H3 optical density value by the total histone H3 optical density value derived independently from the same sample. All samples within each patient set were normalized to the level of histone H3 acetylation from the pretreatment sample of that patient, which was assigned a value of 1.

RESULTS

Patient Characteristics and Treatment Administration

Seventy-six patients were enrolled onto the study and 73 received at least one dose of oral SAHA. Three patients did not receive the study drug due to the development of brain metastasis in one and rapid disease progression in two patients. The most common tumors were mesothelioma (n = 13) and non-Hodgkin's lymphoma (n = 12). Patient characteristics are described in Table 1. Seventy-eight percent of all patients had received two or more prior systemic therapies. The majority of hematologic

Table 1. Patient Characteristics (N = 73)

Characteristic	Solid Tumors (n = 50)	Hematologic Malignancies (n = 23)
Age, years		
Median	60	59
Range	25-78	20-79
Sex		
Male	34	16
Female	16	7
Primary tumor type		
Mesothelioma	13	
Prostate	7	
Urothelial	7	
Thyroid	6	
Renal	6	
Breast	2	
Lung	2	
Adrenal cortical	1	
Germ cell	1	
Laryngeal	1	
Melanoma	1	
Paraganglioma	1	
Skin	1	
Cervical	1	
Hodgkin's lymphoma		7
Non-Hodgkin's lymphoma		
Diffuse large B-cell		7
Small lymphocytic		1
Mantle cell		2
Cutaneous T-cell		1
Peripheral T-cell		1
Myeloma		2
Acute myeloid leukemia		1
Myelodysplastic syndrome		1
No. of prior systemic therapies: chemotherapy, or biologic therapy, or both		
None	1	0
One	14	1
Two	11	4
Three or more	24	18

malignancy patients had received three or more prior systemic therapies (n = 18; 78%). Seventy-three patients received a total of 416 treatment cycles. The median number of treatment cycles was two (range, one to 37+ cycles), which was the same in patients with solid tumor or hematologic malignancies. Twenty-two patients (solid tumor, n = 16; hematologic malignancy, n = 6) completed four or more treatment cycles. Eight completed 12 or more treatment cycles and four patients are still on study with the longest treatment duration exceeding 37 cycles in two patients.

Fifty-six patients (77%) were discontinued from the study because of progressive disease (solid tumor, n = 40; hematologic malignancy, n = 16). Ten patients (14%) were discontinued because of adverse event (solid tumor n = 7; hematologic malignancy n = 3), including one patient with widely metastatic mesothelioma who died of infection without neutropenia during the second week of treatment. The death was considered unlikely to be caused by the study drug. Three patients with hematologic malignancies were removed from the study for protocol violation, noncompliance, and patient withdrawal of consent.

DLT and MTD

The number of patients and DLT for each dose level for the solid tumor and hematologic patients are listed in Table 2. Most of the patients (n = 67, 92%) were treated at the MTD or above. The DLTs were predominantly anorexia, dehydration, diarrhea, and fatigue. The MTD for continuous daily dosing for hematologic and solid tumors was 400 mg qd or 200 mg bid, and for solid tumors 300 mg bid × 3 consecutive days per week. The dosing schedule did not appear to have a major effect on the pattern of DLTs and at the doses that exceeded the MTD; the frequency of DLT increased but the pattern and severity remained the same. At the 400 mg bid dose level, six of nine patients (hematologic and solid tumors) developed DLT in the first cycle of therapy and four of six DLTs occurred in 14 days or less after starting the oral SAHA. The median time to resolution of the DLTs was 7 days (range, 3 to 10 days).

Safety and Tolerability

The most common drug-related adverse events (Table 3) were constitutional (fatigue), gastrointestinal (anorexia, nausea, diarrhea, and vomiting), metabolic (hyperglycemia, and hypocalcemia), and hematologic (anemia and thrombocytopenia). Grade 4 events occurred in nine patients (solid tumor, three patients; hematologic malignancy, six patients), most of which were hematologic: anemia (n = 4), neutropenia (n = 1), thrombocytopenia (n = 1), hyponatremia (n = 1), elevation in creatine phosphokinase (CPK) (n = 1), and infection (n = 1). Overall, a higher incidence of grade 3 or 4 thrombocytopenia (21% *v* 36%) and grade 3 dehydration (21% *v* 36%) was observed in the twice-a-day continuous dosing schedule, more so in hematologic patients than in solid tumor patients. There were no grade 3 or 4 hematologic toxicities seen in the twice-a-day × 3 days-per-week–treated solid tumor patients, but an increase in the incidence of severe fatigue was noticed when compared with continuous twice daily and the once daily regimens (37% *v* 28% *v* 17%, respectively). More patients with hematologic malignancies experienced thrombocytopenia (87%) than solid tumor patients (44%), and thrombocytopenia was more severe in those patients. Grade 3 thrombocytopenia occurred in six solid tumor patients (12%) and eight patients with hematologic malignancies (35%), most of which (79%) resolved to grade 2 within 7 days. Bone marrow biopsies in two patients with grade 3 or 4 thrombocytopenia suggested that the most probable cause of thrombocytopenia was maturation arrest. Grade 3 neutropenia occurred in three hematologic patients and grade 4 in one, all of which resolved to grade 2 or less without intervention. No patients had neutropenic fever or discontinued therapy because of neutropenia. Fatigue was more common at the higher dose levels on the twice-a-day dosing regimens but was reversible within 3 to 7 days. Mild to moderate GI

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Table 2. DLT and Dose Escalation

		Solid Tumor (N = 50)		Hematologic Malignancy (N = 23)	
Dose Level	Dosing Regimen	No. of Patients	DLT	No. of Patients	DLT
1	200 mg qd	6*	None	—	—
2	400 mg qd	5†	None	11‡	Dehydration/diarrhea (n = 1) Dehydration/diarrhea/fatigue (n = 1)
3	400 mg bid	6	Dehydration/diarrhea (n = 1) Fatigue (n = 1) Thrombocytopenia (n = 1)	3	Anorexia (n = 1) Dehydration (n = 1) Anorexia/dehydration (n = 1)
4	600 mg qd	4	Anorexia/dehydration/fatigue/ nausea (n = 1)	3	Dehydration/diarrhea (n = 1) Diarrhea (n = 1)
5	200 mg bid	4	None	6	Anorexia (n = 1)
6	300 mg bid	6	Elevated ALT/AST (n = 1) Anorexia/fatigue (n = 1) Fatigue (n = 1)	—	—
7	300 mg bid × 3 days/week	13§	None	—	—
8	400 mg bid × 3 days/week	6	Fatigue (n = 2) Dehydration/nausea/vomiting (n = 1)	—	—

Abbreviations: DLT, dose-limiting toxicity; qd, every day; bid, twice a day.
*Three patients removed for early progression of disease (POD) during first 4 weeks.
†One patient removed for early POD, and one additional patient treated at this dose level.
‡No DLTs observed in the first five patients enrolled; six additional patients enrolled at the maximum tolerated dose (MTD), and two developed DLTs.
§Ten additional patients treated at the MTD.

symptoms of anorexia, diarrhea, nausea, and vomiting were common and antiemetics and antidiarrheal medications were able to control symptoms. Anorexia was associated with gustatory changes in 8% of the patients.

Twenty-five patients (34%) reported mild to moderate dyspnea without other associated cardiopulmonary symptoms or new abnormalities on the chest x-ray or ECGs. Serial ECGs showed nonspecific ST and QT changes but no consistent patterns were identified. No patients were found to have cardiac arrhythmias, new onset angina, or other cardiac toxicities.

Pharmacokinetics

PK parameters and mean plasma concentration time curves from patients treated with 200, 400, and 600 mg of SAHA are presented in Table 4 and Figure 1. The pharmacokinetics of SAHA after oral administration of a single dose of SAHA are linear from 200 to 600 mg (Fig 2). The mean apparent half-life ($t_{1/2}$) following oral administration (range, 91.6 to 127 minutes) was longer than the mean apparent $t_{1/2}$ following intravenous administration of the oral equivalent doses (range, 34.7 to 42.4 minutes). The estimated bioavailability of SAHA at doses of 200 and 400 mg administered during the fasting state was 43%. Exploratory studies in the fasting and nonfasting (fed) state suggest that oral administration of SAHA with food does not appear to substantially alter the rate or extent of absorption. PK parameters obtained in four patients after 6 months or more of therapy were similar to baseline values.

Table 3. Ten Most Common Drug-Related Toxicities in 73 Patients (all cycles, highest grade per event per patient)

	Solid Tumors (n = 50)		Hematologic Malignancy (n = 23)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Hematologic				
Anemia	36	5	10	7
Thrombocytopenia	16	6	11	9
Nonhematologic				
Anorexia	26	4	10	4
Diarrhea	19	2	12	7
Elevated serum creatinine	28	1	19	0
Fatigue	32	14	16	5
Hyperglycemia	37	6	17	3
Hypocalcemia	20	2	12	1
Nausea	36	3	15	0
Vomiting	23	2	7	1

Correlative Studies

Histone acetylation was evaluated by Western blot or ELISA on histones isolated from PBMNCs. Histone samples were isolated from PBMNCs from 50 patients, and accumulation of acetylated histone H3 (AcH3) was observed at 2 hours after oral ingestion of SAHA consistently in all dose cohorts (Fig 3). As the dose of oral SAHA was increased from 200 to 600 mg, the duration that an accumulation of AcH3 was observed increased from 4 to 10 hours. An accumulation of AcH3 in PBMNCs was consistently observed at all dose levels after 3 weeks of oral SAHA. Two patients that remained on study > 6 months had repeat analysis of the AcH3 (Fig 4). An increase in accumulation of AcH3 was observed in patients on prolonged treatment with oral SAHA.

Antitumor Activity

Twenty-two patients (30%) remained on study for 4 to 37+ months (Table 5). Of these 22 patients, there was one

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Table 4. Pharmacokinetic Parameter for SAHA After Oral Administration

Day	Parameter*	200 mg qd	200 mg bid	300 mg bid	400 mg qd	400 mg bid	600 mg qd
1 (intravenous)	No. of patients	6	NA	NA	8	6	NA
	C_{max}, ng/mL	1,088 ± 567			2,306 ± 1,099	2,184 ± 1,253	
	T_{max}, min	60			60	60	
	AUC_{0-t}, min-ng/mL	105,300 ± 64,224			214,132 ± 133,211	201,020 ± 105,654	
	AUC_{∞}, min-ng/mL	124,518 ± 69,943			163,202 ± 27,794	219,588 ± 107,344	
	$T_{1/2}$, min	34.7 ± 13.4			42.4 ± 15.5	38.4 ± 9.2	
	CL/F, mL/min	2,134 ± 1,283			2,513 ± 439	2,231 ± 1,153	
	Vz/F, L	89.2 ± 23.1			150 ± 51.5	117 ± 51.8	
8 (fasted)	No. of patients	6	10	6	10	6	6
	C_{max}, ng/mL	304 ± 150	301 ± 152	263 ± 100	658 ± 439	349 ± 127	804 ± 397
	T_{max}, min	135	120	53	106	150	90
	AUC_{0-t}, min-ng/mL	43,426 ± 28,400	49,466 ± 38,766	41,489 ± 22,099	101,854 ± 105,570	66,439 ± 15,087	166,555 ± 86,736
	AUC_{∞}, min-ng/mL	40,393 ± 23,046	74,374 ± 74,914	39,730 ± 23,694	161,443 ± 169,849	77,334	139,370 ± 71,002
	$T_{1/2}$, min	91.6 ± 27.2	122 ± 33.8	93.5 ± 25.2	88.9 ± 20.5	100	127 ± 64.2
	CL/F, mL/min	5,987 ± 2,790	4,699 ± 2,994	11,006 ± 7,994	4,409 ± 2,682	5,172	5,418 ± 3,387
	Vz/F, L	853 ± 607	834 ± 617	1,528 ± 1,147	531 ± 346	748	889 ± 444
9 (fed)	No. of patients	6	10	6	9	6	6
	C_{max}, ng/mL	279 ± 151	295 ± 160	297 ± 111	667 ± 696	455 ± 158	685 ± 277
	T_{max}, min	120	150	105	90	105	150
	AUC_{0-t}, min-ng/mL	41,686 ± 24,516	52,074 ± 34,025	51,572 ± 18,183	134,292 ± 181,574	99,293 ± 60,407	174,322 ± 72,414
	AUC_{∞}, min-ng/mL	56,001 ± 37,054	48,120 ± 17,518	48,868 ± 21,839	199,874 ± 252,665	121,970 ± 99,787	236,094 ± 150,007
	$T_{1/2}$, min	70.5 ± 12.9	91.9 ± 39.3	97.8 ± 38.2	135 ± 109	100 ± 34.4	111 ± 58.2
	CL/F, mL/min	5,776 ± 5,256	4,518 ± 1,243	7,410 ± 4,069	3,945 ± 2,086	5,096 ± 3,657	3,184 ± 2,023
	Vz/F, L	522 ± 373	621 ± 359	1,016 ± 476	885 ± 1,138	616 ± 265	424 ± 56
22-30	No. of patients	3	7	5	7	2	3
	C_{max}, ng/mL	233 ± 88.8	263 ± 76.3	263 ± 89.9	446 ± 105	268 ± 78.4	334 ± 160
	T_{max}, min	45	45	60	90	195	120
	AUC_{0-t}, min-ng/mL	33,333 ± 23,678	39,634 ± 13,170	32,658 ± 9,714	65,324 ± 19,435	65,740 ± 29,677	49,602 ± 1,945
	AUC_{∞}, min-ng/mL	†	43,511 ± 14,009	30,759 ± 8,942	92,625 ± 8,461	88,106	75,489
	$T_{1/2}$, min	†	78 ± 46.9	57.6 ± 44.8	98 ± 31.3	43.4	685
	CL/F, mL/min	†	5,129 ± 2,149	10,400 ± 3,008	4,337 ± 396	4,540	7,948
	Vz/F, L	†	513 ± 207	963 ± 1,016	604 ± 140	284	7,851
> 6 months	No. of patients	NA	1	NA	NA	3‡	NA
	C_{max}, ng/mL		247			358 ± 67	
	T_{max}, min		127			80	
	AUC_{0-t}, min-ng/mL		33,949			47,883 ± 9,185	
	AUC_{∞}, min-ng/mL		34,640			47,904 ± 9,175	
	$T_{1/2}$, min		76			79 ± 30	
	CL/F, mL/min		5,890			8,540 ± 1,520	
	Vz/F, L		650			1,000 ± 450	

Abbreviations: SAHA, suberoylanilide hydroxamic acid; qd, every day; bid, twice a day; NA, not applicable; C_{max}, maximum concentration; T_{max}, time to maximum concentration; AUC, area under the curve; $T_{1/2}$, half-life; CL/F, clearance; Vz/F, volume of distribution.

*Mean ± SD except for T_{max}, for which the median is reported. Individual patients are reported if n ≤ 2.

†Parameter could not be calculated

‡At the time of pharmacokinetic studies, one patient was on 400 mg bid and two patients were dose reduced to 400 mg daily.

complete response (CR) in a patient with transformed diffuse large B-cell lymphoma with normalization of the positron emission tomography scan and resolution of the bone marrow involvement for 17 months, and three partial responses (PRs) were noted in the following patients: de novo diffuse large B-cell lymphoma, laryngeal cancer (n = 1), and papillary thyroid cancer (n = 1). Two unconfirmed partial responses were observed in patients with metastatic mesothelioma. Stable disease was seen at all dose levels but confirmed CRs and PRs were only seen at 400 mg bid and 600 mg daily dose levels. An improvement in tumor-related pain and dyspnea was observed in patients with laryngeal cancer and mesothelioma who had tumor regression. Prolonged disease stabilization was also seen in patients with renal cell carcinoma and thyroid cancer with minor objective tumor regression. There were six patients with metastatic thyroid cancer (four poorly differentiated papillary, one Hürthle cell, and one medullary) maintained on oral SAHA for a median of 27 months (range, 12 to 37+ months). Three papillary thyroid patients had radioactive iodine (RAI) scans performed post-therapy and one had an improvement in the RAI scan post-therapy with oral SAHA.

DISCUSSION

This study demonstrates that oral SAHA can be administered safely for prolonged durations at doses that inhibit HDAC activity, has linear pharmacokinetics with good bioavailability, and has a broad range of antitumor activity. This study defined a once daily (400 mg qd), twice daily (200 mg bid), and a twice daily for 3 consecutive days every week (300 mg bid) dosing schedule that could be used safely in future studies. Fatigue, anorexia, dehydration, and diarrhea were the DLTs observed across the three dosing schedules. The DLTs and the doses at which DLTs occurred were similar between patients with hematologic malignancies

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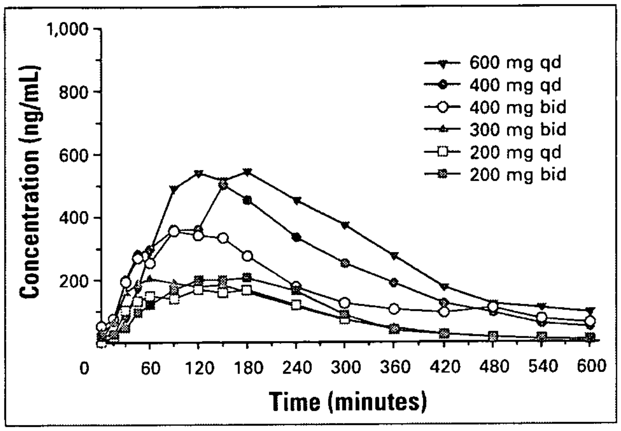

Fig 1. Mean plasma concentrations of suberoylanilide hydroxamic acid on cycle 1/day 9 after oral administration of 200 mg every day (qd) or twice a day (bid), 300 mg bid, 400 mg qd or bid, or 600 mg qd under fed conditions.

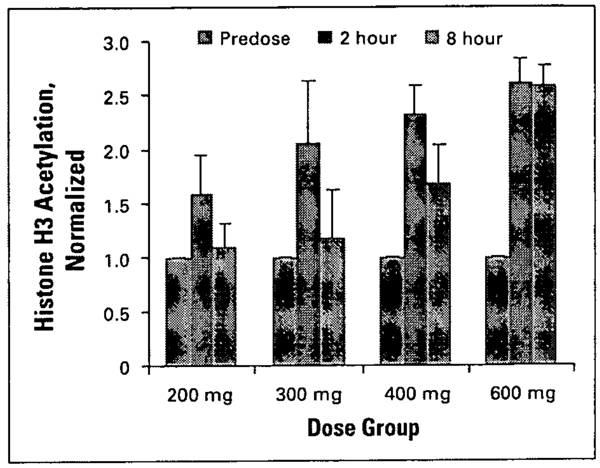

Fig 3. Average histone H3 acetylation by dose group. Histones were isolated at the times indicated following ingestion of suberoylanilide hydroxamic acid and enzyme-linked immunosorbent assays performed as described in Patients and Methods. The number of patients analyzed in the 200-, 300-, 400-, and 600-mg groups was five, three, seven, and three, respectively. Each determination was performed in triplicate and the error bars represent the standard error of the mean.

and solid tumor patients. This is in contrast to the intravenous study of SAHA that showed that myelosuppression limited the dose escalation in patients with hematologic malignancies and there was a three-fold difference in the MTD between solid tumor and hematologic patients.[9] The more extensive prior therapy hematologic patients received may account for these differences. The DLTs with oral SAHA were not related to prior therapy or the type of underlying malignancies but remained relatively unpredictable within treatment cohorts. The fatigue could occur rapidly and was associated with anorexia, dehydration, diarrhea, and a feeling of dyskinesia. Once the oral SAHA was discontinued, these toxicities resolved quickly within 4 to 7 days. The etiology of the fatigue is not known, but their rapid resolution after withdrawing the SAHA suggests a readily reversible metabolic process.

In this study, the majority of the patients were treated at MTD or above and once on a tolerable dose, patients could be treated for prolonged periods of time, in some cases for over 2 years without loss of the biologic effect. Chronic adverse effects (fatigue, renal insufficiency, and weight loss) seen in long-term treated patients were generally mild to moderate and were reversible on discontinuation of the study drug, suggesting that chronic administration of SAHA is feasible and safe.

The altered PK profile of oral SAHA as compared with IV SAHA is likely to have contributed to the differences in toxicity, prolonged biologic effect, and the clinical outcomes in patients. As with the IV formulation of SAHA, the extent of exposure after oral SAHA administration was linear in dose ranges from 200 to 600 mg.[9] Peak concentrations were substantially lower after oral administration, but there was a two- to three-fold increase in the apparent half-life when compared to the IV administration. Oral SAHA plasma concentrations could be detected

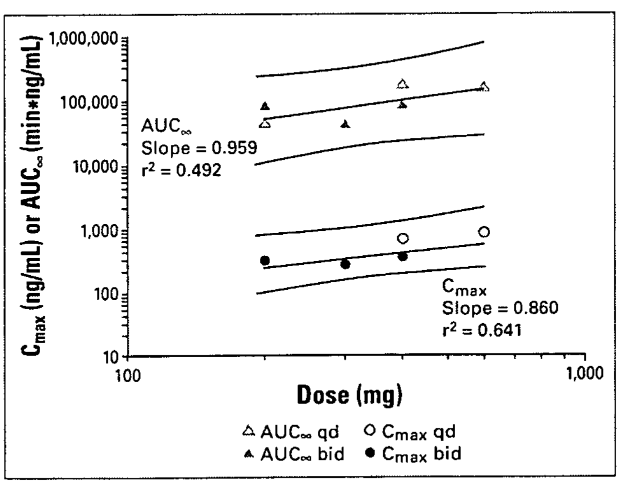

Fig 2. Relationship between area under the curve to infinity (AUC_∞) and maximum concentration (C_{max}) and dose on cycle 1/day 8 after administration of suberoylanilide hydroxamic acid under fasting conditions. qd, every day; bid, twice a day.

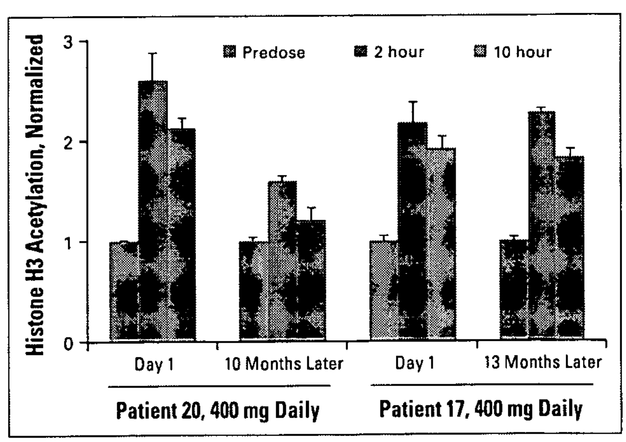

Fig 4. Long-term evaluation of peripheral-blood mononuclear cells for histone H3 acetylation in patients.

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Table 5. Patients on Study ≥ 4 Months

Tumor	SAHA dose (mg)	No. of Prior Systemic Therapy	Best Response	On-Study Duration (months)
Solid tumor				
Thyroid	200 qd	1	SD	22
Renal cell*	400 qd	3	SD	4
Urothelial	200 bid	3	SD	10
Thyroid	200 bid	1	SD	28
Thyroid	300 bid	1	SD	28+
Mesothelioma	300 bid x 3 days/week	1	SD	8+
Mesothelioma	300 bid x 3 days/week	5	PR††	8
Mesothelioma†	300 bid x 3 days/week	2	SD	5
Mesothelioma	300 bid x 3 days/week	0	SD	5
Mesothelioma	300 bid x 3 days/week	1	PR††	6
Mesothelioma‡	400 bid x 3 days/week	1	SD	10
Thyroid	400 bid x 3 days/week	1	SD	12
Laryngeal§	400 bid	1	PR	10
Renal cell¶	400 bid	4	SD	37+
Thyroid‖	400 bid	1	PR	34
Thyroid**	400 bid	2	SD	37+
Hematologic malignancy				
Hodgkin's lymphoma	400 qd	2	SD	10
Hodgkin's lymphoma	400 qd	8	SD	5
DLBCL (de novo)	600 qd	4	PR	5
DLBCL (transformed)	200 bid	3	SD	8
Cutaneous T-cell	200 bid	5	SD	4
DLBCL (transformed)	400 bid	8	CR	13

Abbreviations: SAHA, suberoylanilide hydroxamic acid; qd, every day; SD, stable disease; bid, twice a day; PR, partial response; DLBCL, diffuse large B-cell lymphoma; CR, complete response.
*400 qd for 7 weeks; 200 qd for 9 weeks.
†300 bid × 3 days/week for 4 weeks; 200 bid × 3 days/week for 14 weeks.
‡400 bid × 3 days/week for 33 weeks; 300 bid for 7 weeks.
§400 bid for 2 weeks; 400 qd for 36 weeks.
¶400 bid for 12 weeks; 400 qd for 136+ weeks.
‖400 bid for 4 weeks; 400 qd for 132 weeks.
**400 bid for 93 weeks; 400 qd for 53+ weeks.
††Unconfirmed.

at 10 hours postingestion at the higher dose levels while the plasma concentration of IV SAHA at similar doses were not detectable after 4 to 6 hours. This would suggest an absorption-rate–limited drug disposition in the GI tract and possibly hepatic recirculation to the GI tract.

As previously shown with intravenous SAHA using Western blot analysis, an increase in histone acetylation in PBMNCs was observed 2 hours post-therapy consistently in all patients evaluated and could persist for up to 10 hours after a single 400 mg or higher dose. This biologic effect paralleled the prolonged plasma concentrations of oral SAHA. An increase in acetylated histones was detected in patients who were on study for 6 months or longer, suggesting there is a sustained biologic effect over time.

Tumor regression and stable disease were observed in a wide range of patients with solid tumors and lymphomas. Four patients had confirmed CR and PR that occurred at the 400 mg bid and 600 mg qd dosing schedules and three of the 4 patients were treated on a twice daily regimen. This suggests that higher doses of SAHA with more prolonged daily exposure may be required for tumor regression. However, prolonged stable disease with minor tumor regression was seen at all dose levels and dosing schedules. Of particular interest was the clinical activity observed in patients with lymphoma and malignant mesothelioma. One CR and one PR were seen in patients with diffuse large B-cell lymphoma and two unconfirmed partial responses in patients with mesothelioma. Preclinical data suggest that HDACs play a critical role in the malignant transformation and cell differentiation[13-16] in these tumors and provides a rationale for developing HDAC inhibitors in these diseases. Of note in this study, 30% of the heavily pretreated patients had stable disease for 4 or more months and five patients remained on therapy for more than 2 years. Four of the five of these long-term patients had metastatic thyroid cancer which may have a more indolent course; however, all had objective disease progression before study entry. Thyroid cancer patients were initially accrued to this trial based on the data from Kitazono et al, showing that the HDAC inhibitor, depsipeptide, led to an increase in the expression to the Na+/iodine symporter that could result in an increase ^{125}I uptake in thyroid cells.[17] This could possibly lead to resensitizing RAI-refractory patients to RAI.[17,18] In this study, one patient did have an increase in the RAI scan post-therapy. Other plausible mechanisms for disease stabilization need to be investigated in thyroid cancer, since one patient with medullary thyroid cancer was also maintained on therapy for over 2 years.

In summary, this first study of oral SAHA demonstrated that SAHA could be administered safely for prolonged periods of time while maintaining the biologic effect of the drug and exhibiting a broad range of antitumor activity at multiple dose levels and dosing schedules. Future studies need to define the optimal dosing schedule and elucidate the biologic consequences of HDAC inhibition in patients. Currently, there are multiple phase II studies in patients with hematologic and solid tumor malignancies that are exploring the efficacy of daily and twice daily schedules that will help to determine the most optimal dosing regimen.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Employment: Judy H. Chiao, Aton Pharma; Paul Secrist, Aton Pharma; Victoria M. Richon, Merck & Company.

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Modulation of oral bioavailability of anticancer drugs: from mouse to man

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Abstract

Oral bioavailability of many anticancer drugs is poor and highly variable. This is a major impediment to the development of new generation drugs in oncology, particularly those requiring a chronic treatment schedule, a.o. the farnesyltransferase inhibitors. Limited bioavailability is mainly due to: (1) cytochrome P450 (CYP) activity in gut wall and liver, and (2) drug transporters, such as P-gp in gut wall and liver. Shared substrate drugs are affected by the combined activity of these systems. Available preclinical in vitro and in vivo models are in many cases only poorly predictive for oral drug uptake in patients because of a.o. interspecies differences in CYP drug metabolism and intestinal drug-transporting systems. Clearly, novel systems that allow reliable translation of preclinical results to the clinic are strongly needed. Our previous work, also using P-gp knockout (KO) mice, already showed that P-gp has a major effect on the oral bioavailability of several drugs and that blockers of P-gp can drastically improve oral bioavailability of paclitaxel and other drugs in mice and humans (Schinkel et al., Cell 77 (1994) 491; Sparreboom et al., Proc. Natl. Acad, Sci. USA 94 (1997) 2031; Meerum Terwogt et al. Lancet 352 (1998) 285). This work revealed, however, that apart from P-gp other drug-transporting systems and CYP effects also determine overall oral drug uptake. The taxanes paclitaxel and docetaxel are considered excellent substrate drugs to test the concept that by inhibition of P-gp in the gut wall and CYP activity in gut wall and/or liver low oral bioavailability can be increased substantially. In current studies we focus on the development of chronic oral treatment schedules with these drugs and on other drug transport systems that may play a significant role in regulation of oral bioavailability of other classes of (anti-cancer) drugs. The current review paper describes the background and summarizes our recent results of modulation of oral bioavailability of poorly available drugs, focused on drug transport systems and CYP in gut wall and liver. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Paclitaxel; Docetaxel; Oral administration; Bioavailability; P-Glycoprotein; Cytochrome P450

Introduction

Oral applicability is crucial for anticancer agents which should be applied chronically to become effective. This concerns in particular new cytostatic agents with novel mechanisms of action, such as interference with signal transduction pathways and the angiogenesis process (DeMario and Ratain, 1998). Adequate oral bioavailability is also advantageous for more classical cytotoxic agents for which the time-period of exposure is a major determinant of their anticancer activity (Huizing et al., 1997a,b). Unfortunately, the great majority of currently available cytostatics cannot be applied orally, because of low and highly variable oral bioavailability (DeMario and Ratain, 1998). Typical examples are the clinically important taxanes paclitaxel and docetaxel (Meerum Terwogt et al., 1998; Sparreboom et al., 1997). Oral administration of a number of other widely applied or extensively investigated anticancer drugs, such as the camptothecins topotecan, GI147211 (GG211), 9-aminocamptothecin, topoisomerase

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II inhibitors etoposide and teniposide, the anthracyclines doxorubicin, epirubicin and daunorubicin, all *Vinca* alkaloids, ifosfamide and mitoxantrone is also hampered by large interpatient variability and low bioavailability (DeMario and Ratain, 1998; Gerrits et al., 1997a,b; Schellens et al., 1996). In view of the narrow therapeutic window, this leads to under- and overexposure. Consequently, this results in low likelihood of activity in some and unpredictable severe toxicity in other patients.

Two major mechanisms can explain the variable oral pharmacokinetics, which are (1) high affinity for drug-transporters, such as P-glycoprotein (P-gp) that is expressed in the intestinal epithelium and directed towards the gut lumen and (2) high extraction of the drug by extensive metabolism in the gut wall and/or liver, during 'first-pass', i.e., prior to entering the systemic circulation (DeMario and Ratain, 1998; Sparreboom et al., 1997; Van Asperen et al., 1999). The combined activity of drug-transporters and metabolic enzymes of the cytochrome P450 system (CYP) explains the low and variable bioavailability of a range of shared substrate drugs. Furthermore, extensive and variable biotransformation by CYP can result in formation of one or more metabolites with pharmacological activity which may be equal to or even higher than that of the parent drug. Lack of or inadequate information about the involvement of these mechanisms during the early process of anticancer drug development results in inadequate exposure of patients at low dose-levels and unpredictable severe toxicity in other patients at higher dose-levels. This makes the development process of novel drugs slow, inefficient, patient-unfriendly and extremely costly. A more rational approach based on preclinical concepts and results is urgently needed.

Despite the widespread use of rodent and human microsomes, liver slices and cDNA-based vector systems, there are not yet optimal preclinical in vitro or in vivo models or insights available that enable accurate extrapolation of preclinical data about oral pharmacokinetics to the clinic. Sources of inaccuracy may include interspecies variation in activity of CYP isoforms (Lin, 1998), involvement of active transport by P-gp (Van Asperen et al., 1999) or other as yet unidentified transporters and presence of extrahepatic metabolism, in particular in the gut wall (Lin, 1998). Clearly, there is a strong need for development of tools and mechanistic insights that would allow adequate prediction of the pathways of biotransformation, extent of first-pass metabolism, occurrence of drug–drug interactions and oral pharmacokinetics of new anticancer agents during the early preclinical phase of drug development. These insights could then be used to optimize the oral pharmacokinetics, by selective inhibition of drug metabolism pathways and/or drug-transport processes. These tools could be used as a high throughput and early screening filter for fast tracking of drug development in oncology and possibly other therapeutic areas.

Assessment of the clinical pharmacology of orally applied anticancer agents

Traditionally, in contrast to almost any other therapeutic area, the clinical pharmacology of anticancer agents is first investigated after intravenous (i.v.) administration. This approach should change rapidly, because the novel mechanisms applied for inhibition of tumor growth, metastatic spread, the angiogenesis process or chemo-prevention dictate that anticancer drugs are to be applied chronically almost invariably on a daily basis. For this approach only the oral route is appropriate (DeMario and Ratain, 1998).

Besides the low affinity for drug-transporters and drug-metabolizing enzymes, anticancer drugs should have additional important characteristics favoring oral application. Oral drugs should be stable at the low gastric pH, have a reproducible and good pharmaceutical dissolution profile and adequate hydrophilic/lipophilic balance to cross the intestinal epithelial membrane. Furthermore, they should not induce significant gastrointestinal toxicity, such as nausea, vomiting, loss of appetite or diarrhea that would limit continued oral administration or result in poor compliance (DeMario and Ratain, 1998).

In order to determine the oral applicability of anticancer drugs in patients a great number of time-consuming clinical and pharmacological investigations are required. This includes assessment of the absolute oral bioavailability and interpatient variation in systemic exposure upon oral versus i.v. administration (Herben et al., in press, a,b). The intra-patient variation in exposure after repeated oral administration needs to be determined in relation to the toxicity pattern (Gerrits et al., 1999). The maximum tolerated dose (and if possible the minimal effective dose) must be determined in, often very time-consuming, dose-escalating studies which for safety reasons have to start at low dose levels (Gerrits et al., 1997a,b). The mass balance of the oral formulation has to be determined. This means that a quantitative balance should be assessed of the percentage of the administered drug that leaves the body by the renal and fecal route as parent drug and as metabolites. It occurs frequently that new metabolites are detected in these clinical investigations, because current preclinical models do not accurately predict biotransformation of the drug in patients. Consequently, these metabolites should be chemically synthesized, because additional toxicology studies in representative animal species must be executed to investigate the toxicity of these compounds (CPMP/ICH/300/95, Website). Furthermore, a food–drug interaction study should be carried out to determine whether the patients should need additional instructions for drug intake to prevent inadequate and variable systemic exposure induced by food-components (Herben et al., in press, a,b). Also, appropriate drug–drug interaction studies should be carried out of combinations of drugs that interfere with the physiological gastric pH or the rate of

gastric emptying and drugs that potentially interfere with drug transport (P-glycoprotein) and drug metabolism (CYP, glucuronidation).

At present, the relatively new oral anticancer drug development process is largely empirical. Consequently, it is extremely slow and inefficient. It may take as long as 2–4 years before all necessary early clinical studies have been carried out as a basis for investigation of the activity of the oral drug in phase II studies. Clearly, there is a need to develop model systems to enable accurate prediction whether one or more of the outlined barriers exist for the drug under investigation and whether non-toxic methods should be applied, as for example inhibitors of drug metabolism and/or drug-transport, to optimize oral drug pharmacokinetics.

Cytochrome P450 (CYP) and anticancer drug biotransformation

CYP is the main oxidative drug metabolizing enzyme system, also for anticancer drugs. CYP is highly expressed in the liver and gut wall, but the relative contribution of the gut wall to biotransformation is currently unknown. CYP consists of approximately 40 P450s (Nelson et al., 1993; Nelson, 1998), but there is a relatively small number of seven enzyme families that is responsible for approximately 90% of oxidation of drugs and carcinogens (Guengerich, 1996). The main CYP isoforms are 1A (1A1 and 1A2), 2A (2A6), 2B (2B6), 2C (2C8, 2C9, 2C18, polymorphic 2C19), 2D (polymorphic 2D6), 2E (2E1) and 3A (3A4 and 3A5), which have partly overlapping substrate selectivities (Lin and Lu, 1998; Smith et al., 1994). CYP3A and CYP2C are the most abundant subfamilies in the liver, accounting for 30 and 20% of the total CYP, respectively. Of these, CYP3A4 is the most abundant isoform comprising approximately 25% of the total CYP. CYP3A4 is very important in drug metabolism, accounting for at least 60% of total biotransformation of compounds (Lin and Lu, 1998). Importantly, there is a striking overlap between substrates for P-gp and CYP3A4 (Wacher et al., 1995, 1996). Many anticancer agents are extensively metabolized by CYPs, mostly isoform 3A4, e.g., the taxanes paclitaxel and docetaxel, the *Vinca* alkaloids, anthracyclines, several camptothecin derived topoisomerase I inhibitors, epipodophyllotoxins as etoposide and teniposide and others. Also, a number of novel anticancer agents as yet only known by code names (e.g., ET743, R115777, ABT839) are extensively metabolized by CYP. Anticancer drugs metabolized by CYP3A can have widely divergent chemical structures.

Man has four functional CYP3A genes and proteins, CYP3A3, 4, 5, and 7. CYP3A4 and 5 are present in liver and intestine, CYP3A3 in liver, and CYP3A7 is found in fetal liver (e.g., Guengerich, 1996; Komori et al., 1990; Lown et al., 1994). Of these, CYP3A4 and 5 are most relevant for first-pass metabolism of drugs. Like man, the mouse also has at least four Cyp3a genes, of which two, Cyp3a11 and Cyp3a13, are expressed in adult liver. Cyp3a11 is 5- to 10-fold more abundant in liver than Cyp3a13, and it is also expressed in intestine (Itoh et al., 1994; Yanagimoto et al., 1992, 1994, 1997). Information on the mouse Cyp3a family is still quite limited.

Pharmacologically important multidrug transporters: P-glycoprotein and MRPs

P-Glycoprotein

P-Glycoprotein (P-gp) is a large plasma membrane protein of the ATP binding cassette (ABC) family of transporter proteins (Higgins, 1992). The polypeptide consists of two similar halves, each containing six putative transmembrane segments and an intracellular ATP binding site (Chen et al., 1986; Gros et al., 1986; Hsu et al., 1989; Juliano and Ling, 1976). Using ATP, the human MDR1 P-gp can actively transport a wide range of relatively hydrophobic, amphipathic drugs out of the cell. Compounds transported by P-gp include important anticancer drugs like *Vinca* alkaloids, anthracyclines, epipodophyllotoxins and taxanes, and by lowering the drug concentration at intracellular target sites, P-gp can confer multidrug resistance (MDR) to tumor cells (Gottesman and Pastan, 1993).

A combination of two sets of properties makes for an important role of P-gp in pharmacology: (1) its extremely wide substrate specificity, including many important drugs of widely divergent structures next to the anticancer drugs currently in use, and (2) its presence in many membranes forming essential pharmacological barriers, such as the epithelial apical membrane of the intestine and of the proximal tubules of the kidney, the biliary canalicular membranes of hepatocytes, the luminal membrane of endothelial cells at the blood–brain and blood–testis barriers, and the apical membrane of syncytial trophoblasts of the placenta (Cordon-Cardo et al., 1989; Sugawara et al., 1988; Thiebaut et al., 1987). Given the direction of transport mediated by P-gp, this tissue distribution results in protection against uptake of drugs from the gut into the bloodstream, or from the bloodstream into brain, testis, or fetus, and in active excretion of drugs by the liver and across the gut and kidney proximal tubular epithelia (Lankas et al., 1998; Schinkel et al., 1996; Schinkel, 1997). These putative functions were all observed by us or confirmed in knockout mice lacking Mdr1-type P-gps (Schinkel et al., 1994, 1995).

Unlike humans, that have only one Mdr1-type P-gp, mice have two: Mdr1a and Mdr1b P-gp. Since the substrate-specificity and the combined tissue distribution of

the two murine P-gps are very similar to that of the human MDR1 P-gp, it appears that the murine Mdr1-type P-gps together perform the same biological function(s) as the human MDR1 P-gp (Croop et al., 1989; Devault and Gros, 1990). Thus, in order to understand the functions of the human MDR1 P-gp, we are studying knockout mice lacking Mdr1a and Mdr1b P-gps (reviewed in Herben et al., in press, a,b).

Interestingly, as outlined above, there is a very extensive, albeit not complete, overlap between the drug substrates of the Mdr1-type P-gps and CYP3A4 (Berg-Candolfi et al., 1996; Cupp and Tracy, 1997; Wacher et al., 1995, 1996). CYP3A enzymes are predominantly found in the liver and in the intestinal epithelial cells and can thus perform a very substantial fraction of the first-pass metabolism of orally administered drugs. Since the activity of P-gp in the intestinal epithelium will limit the net rate of entry of orally administered substrate drug into the epithelial cell, and thus also its passage into the liver, this may result in more efficient metabolism of the drug, as the metabolic enzyme(s) will not be saturated as quickly as in the absence of P-gp. Thus, the combined activity of CYP3A and P-gp may be a major determinant of limited and/or variable oral bioavailability of shared substrate drugs (see e.g., Lown et al., 1997a,b; Watkins et al., 1987; Watkins, 1992, 1997).

Many inhibitors for P-gp have been identified, some of which are very effective and have low intrinsic toxicity to organisms, which allows administration at doses sufficient to block the endogenous P-gp activity. Examples are verapamil (Tsuruo et al., 1981), cyclosporin A, PSC833 (Boesch et al., 1991), GG918 (Witherspoon et al., 1996) and LY335979 (Dantzig et al., 1996).

MRPs

Like P-gp, members of the multidrug resistance-associated protein (MRP) family are ABC transporters with the capacity to mediate transmembrane transport of many (conjugated) drugs and other compounds. At least two members of this family, MRP1 (Cole et al., 1992) and MRP2/cMOAT have been demonstrated to affect in vivo pharmacokinetics of a range of drugs (Kartenbeck et al., 1996; Paulusma et al., 1996; Wijnholds et al., 1997). Interestingly, SN38, which is the active metabolite of CPT11, is also transported by MRP2 in vivo (Chu et al., 1997). Currently, no clinically applicable modulation of MRP activity is available.

In vivo pharmacological insights obtained from Mdr1a and Mdr1b P-gp knockout mice

Pharmacological analysis of the Mdr1a and Mdr1b knockout mice generated by us has yielded many fruitful insights: it established that these P-gps are not essential for normal development, viability or reproduction of mice, suggesting that it may be feasible to chronically block P-gp activity with P-gp inhibitors if this is desirable for pharmacotherapeutic applications (Schinkel et al., 1994, 1997).

Using these mice, or a spontaneously occurring Mdr1a mutant mouse strain, we and others have demonstrated that Mdr1-type P-gp has an important role in limiting the penetration of a range of anticancer and other drugs into the brain, testis and fetus (Lankas et al., 1998; Schinkel et al., 1994, 1995, 1996). We further demonstrated that the intestinal P-gp mediates substantial direct trans-epithelial excretion of drugs including paclitaxel (Mayer et al., 1996; Sparreboom et al., 1997), whereas liver P-gp mediates considerable hepatobiliary excretion of, e.g., doxorubicin and other drugs (Smit et al., 1998; Van Asperen et al., 1999). In concert, these P-gp-mediated transport activities can contribute to a more efficient clearance of drug from the circulation (Schinkel et al., 1994; Sparreboom et al., 1997; Van Asperen et al., 1996).

Another striking effect of intestinal Mdr1-type P-gp is its influence on the uptake of orally administered substrate drugs. For instance, we found that the plasma AUC of orally administered paclitaxel is more than 6-fold increased in mdr1a (−/−) mice compared to wild-type mice (Sparreboom et al., 1997). Other groups found similar effects with, e.g., HIV protease inhibitors (Kim et al., 1998). In view of the general importance of oral bioavailability of drugs, including anticancer drugs, we subsequently tested whether efficient P-gp inhibitors such as cyclosporin A and its analogue PSC833 could improve the oral bioavailability of paclitaxel in mice and in patients (Meerum Terwogt et al., 1998; Van Asperen et al., 1997, 1998). Indeed, we found that in mice a 10-fold increase in plasma AUC could be obtained with PSC833 and cyclosporin A (CsA). Comparison with mdr1a (−/−) mouse data suggested that about half of this effect (i.e., 5-fold) could be attributed to P-gp inhibition, and the rest (about 2-fold) to inhibition of another paclitaxel-clearing mechanism, most likely metabolism by mouse Cyp3a family members. In humans, we have observed quantitatively comparable effects on paclitaxel bioavailability upon co-administration of cyclosporin A and paclitaxel (Meerum Terwogt et al., 1998).

Conceptual clinical studies

Results obtained in wild-type and Mdr P-gp knockout mice (see above and Fig. 1A) have been the starting point for two proof of principle studies in patients with solid tumors. In the first study five patients were given a safe oral dose of 60 mg/m^2 of paclitaxel and nine other patients received the same oral dose of paclitaxel combined with one single oral dose of 15 mg/kg of CsA.

For this study the i.v. formulation was given orally to patients. The apparent oral bioavailability of paclitaxel when given without CsA was less than 5% and increased

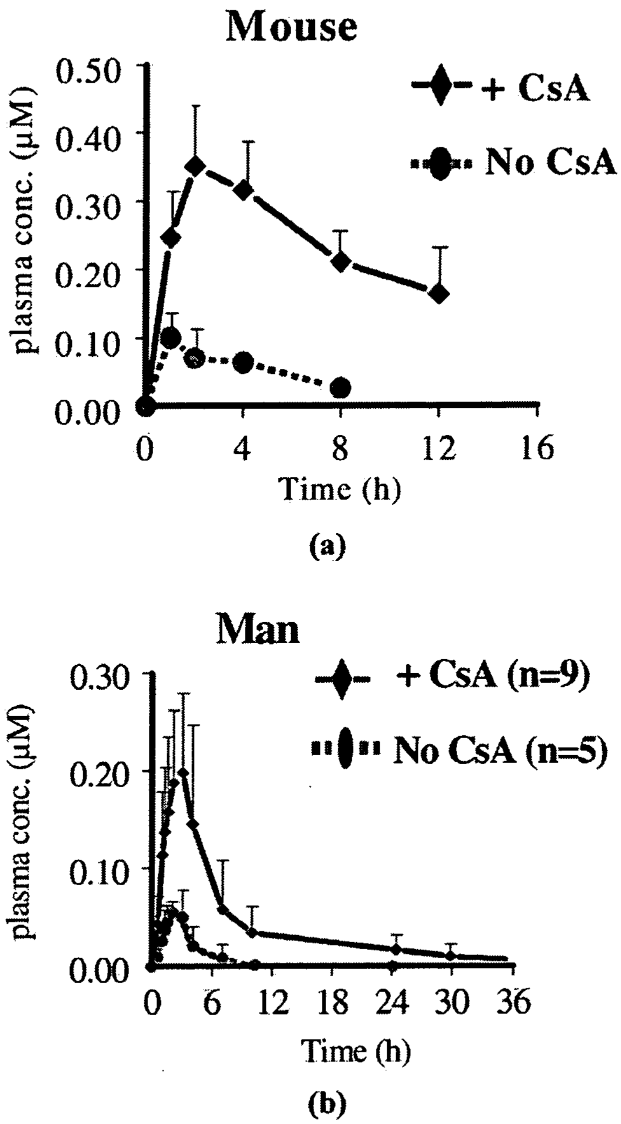

Fig. 1. (A) Effect of a single oral dose of CsA (50 mg/kg) on the systemic exposure or oral paclitaxel (5 mg/kg) in wild-type mice (Van Asperen et al., 1998). (B) Effect of a single oral dose of CsA (15 mg/kg) on the systemic exposure of oral paclitaxel (60 mg/m^2) in patients (Meerum Terwogt et al., 1998).

to 50% when CsA was co-administered (Fig. 1B). These data confirm the preclinical results (Fig. 1A). The oral doses were very well tolerated by the patients and induced no side-effects.

Another major advantage of the oral route was that the solvent Cremophor EL, which is an important component of the i.v. formulation of Taxol®, was not absorbed from the GI tract. Our previous results reveal that Cremophor EL is responsible for the significant hypersensitivity reactions of paclitaxel infusions and of the non-linear pharmacokinetics upon i.v. administration (Sparreboom et al., 1998). Thus the oral route may also be beneficial from a safety point of view. In a dose-ranging study, doses of up to 300 mg/m^2 of oral paclitaxel+CsA were safe and well tolerated (Malingré et al., 1999a–c). In a dose-ranging study of CsA, single doses of 5, 10, 15 and 30 mg/kg resulted in the same maximal effect on the oral pharmacokinetics of paclitaxel. We also performed a mass balance study employing oral paclitaxel+CsA and i.v. paclitaxel in the same patients, but at different occasions. The results reveal that the recovery of parent drug and metabolites is nearly complete at both occasions. After i.v. administration the majority of the dose is excreted as metabolites by the fecal route and after oral administration as parent drug plus metabolites by the same route (Meerum Terwogt et al., 1999). The relative contribution of formation of 3′-*p*-hydroxypaclitaxel was substantially lower after oral than after i.v. administration. This metabolite is formed by CYP3A4, in contrast to the other major metabolite 6α-hydroxypaclitaxel (CYP2C8), indicating inhibition of this metabolic pathway by CsA. Preliminary data from our study reveal that the oral route of paclitaxel results in significant antitumor activity. Phase II studies with this concept, aimed at assessment of the antitumor activity are ongoing.

Subsequently, it was investigated whether an increase in the cyclosporin A dose or fractionated cyclosporin A administration would result in an increase in paclitaxel AUC values (Schellens et al., 1998). Dose-increment of cyclosporin A to 30 mg/kg and changing the schedule to two administrations of 15 mg/kg separated by 2 h did not result in a further increase in the AUC of paclitaxel. Apparently, P-gp inhibition was maximal at a single dose of cyclosporin A of 15 mg/kg. It remained, however, unclear whether cyclosporin A inhibited P-glycoprotein completely. In addition, incomplete distribution of cyclosporin A over the mucosa wall may also have contributed to the possible incomplete P-glycoprotein inhibition by cyclosporin A.

In an attempt to further increase the systemic exposure of orally administered paclitaxel and to determine the dose-limiting toxicity and maximum tolerated dose, dose-escalation of oral paclitaxel was investigated (Malingré et al., 1999a–c, in press). Dose limiting toxicity was reached at the dose level of 360 mg/m^2 and consisted of acute nausea and vomiting. The maximum tolerated dose was then defined at 300 mg/m^2. Pharmacokinetic analysis of oral paclitaxel revealed that dose-escalation of oral paclitaxel from 60 to 300 mg/m^2 resulted in significant increases in the AUC of paclitaxel; however, these increases were moderate and not proportional with increases in dose. It was hypothesized that this non-linear absorption pharmacokinetic behavior of oral paclitaxel was due to the poor aqueous solubility of paclitaxel and consecutive limited dissolution in the gastro-intestinal tract. A similar non-linear pharmacokinetic absorption pattern due to poor aqueous solubility was observed for the oral anticancer drugs etoposide and the platinum complex JM216 (Hande et al., 1993; McKeage et al., 1995).

Based on the non-linear drug absorption a split dose regimen was investigated to achieve a greater overall daily systemic exposure (Malingré et al., 1999a–c). Oral paclitaxel was administered in two doses 7 h apart at dose levels of 2×60, 2×90 and 2×120 mg/m^2. In this study with oral paclitaxel, besides the AUC value, the pharmacokinetic parameter time above the threshold concentration of 0.1 μM (T>0.1 μM) was considered. Previous clinical work has suggested that time above this threshold concentration is related to the activity of the drug (Huizing et al., 1993, 1997a,b). The pharmacokinetic data revealed that bi-daily dosing of oral paclitaxel also shows non-linear absorption pharmacokinetics as was observed after single dose administration of the drug. Comparison with the pharmacokinetic data after single dose administration revealed that fractionated administration of the drug resulted in higher AUC and T>0.1 μM values of paclitaxel. Therefore, a multiple dosing regime may be a realistic option to further increase the systemic exposure after oral administration of paclitaxel.

In a second proof of principle study the same design was applied, but now docetaxel was given orally instead of paclitaxel. The oral bioavailability increased from as low as 8±6% without CsA to almost complete bioavailability (88±36%) in combination with CsA (Richel et al., 1999). Importantly, the interpatient variability in the AUC after oral administration+CsA was of the same order as after standard i.v. administration. The safety of the oral route was very good. This was considered an excellent starting point for clinical phase II studies with the oral concept and this trial has recently been activated in patients with metastatic breast cancer.

Conclusions and future directions

Oral treatment with the taxanes paclitaxel and docetaxel is to be preferred as oral drug administration is convenient to patients, reduces administration costs and facilitates the use of more chronic treatment regimens. In addition, for paclitaxel, circumvention of systemic exposure to the cosolvent Cremophor EL is another advantage of oral therapy. Based on the extensive preclinical research we have shown the feasibility of oral administration of the taxanes in cancer patients by concomitant administration of oral cyclosporin A.

For orally administered paclitaxel at a dose of 60 mg/m^2 a bioavailability of close to 50% was determined. However, true bioavailability of oral paclitaxel might be significantly higher due to the non-linear pharmacokinetics of i.v. paclitaxel. The maximum tolerated dose of oral paclitaxel was determined at 300 mg/m^2. However, because of the non-linear absorption pharmacokinetics of oral paclitaxel and the large amount of parent drug recovered after oral paclitaxel administration at a dose of 300 mg/m^2, administration of lower paclitaxel doses (180 mg/m^2) is considered most appropriate. Fractionated administration of oral paclitaxel appeared to result in an increase in the systemic exposure to paclitaxel compared to single-dose administration and may therefore be a realistic option to increase the systemic exposure after oral administration of paclitaxel.

For orally administered docetaxel at a dose of 75 mg/m^2 a bioavailability of nearly 90% was achieved with an interpatient variability similar to that after i.v. administration. The oral combination was well tolerated. Hence, oral administration of docetaxel is a realistic alternative to i.v. treatment of the drug. Furthermore, oral treatment may facilitate the use of weekly treatment regimens which currently become popular. The activity of weekly oral docetaxel in combination with cyclosporin A is currently investigated in a phase II study in patients with advanced breast cancer.

Finally, the concept of modulation of bioavailability by a P-gp inhibitor may well be applied for other (cytotoxic) drugs that show affinity for the multidrug efflux pump and are associated with poor or moderate oral bioavailability.

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